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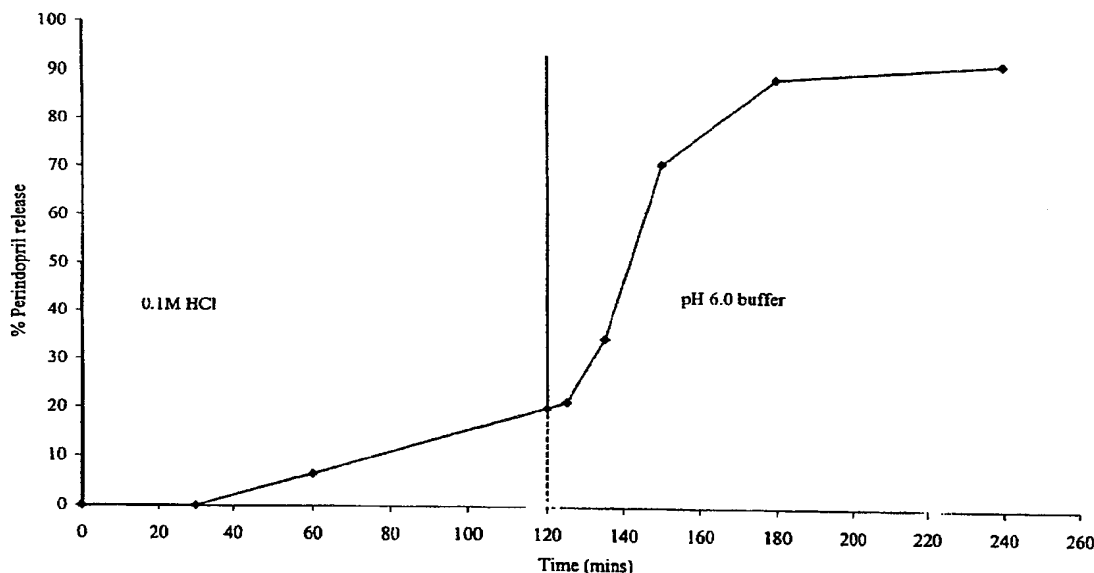
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(54) Title: FLOATING DRUG DELIVERY COMPOSITION



(57) Abstract: There is provided a multiparticulate drug delivery composition adapted for the delivery of a pharmacological agent to the small intestine of a mammal, including drug-containing coated particles, which particles comprise a drug-containing core coated with an enteric polymer that prevents significant release of drug in an acid environment but permits drug release in a more alkaline environment, wherein the particles float when suspended in water.

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FLOATING DRUG DELIVERY COMPOSITION

This invention relates to novel formulations that provide for controlled and delayed drug delivery to the small intestine.

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It is now appreciated that the symptoms and progress of certain diseases is/are not randomly distributed over a 24-hour period. Consequently, in some cases, intervention with a therapeutic agent should not be in a random fashion. Intervention should be timed in such a way as to
10 maximise the effectiveness of the therapeutic agent in treating the disease.

Examples of where this approach may be appropriate include cancer treatment, hypertension and coronary heart disease, treatment of peptic ulceration with H₂-receptor antagonists, the use of non-steroidal anti-
15 inflammatory drugs and glucose regulation in diabetes treatment. For example, in some cases it may be advantageous to provide drug treatment in the early hours of the morning so that optimal plasma levels of drug are reached as the patient wakes up. This approach might be expected to be especially useful for drugs such as anti-inflammatory agents to treat
20 rheumatoid arthritis, or drugs for the treatment of cardiovascular diseases, such as hypertension and angina.

The subject of co-ordinating drug treatment with disease states is known as chronopharmacology and has been reviewed by Lemmer in
25 "*Chronopharmacology, Cellular and Biochemical Interactions*", Dekker, New York (1989). The implications for drug delivery have been the subject of a meeting of the New York Academy of Sciences ("*Temporal Control of Drug Delivery*", *Ann. New York Acad. Sci.* Vol. **618** (1991))

and a workshop in Germany ("*Pulsatile Drug Delivery*", Eds. Gurney *et al.*, Stuttgart, Wiss. Verl. (1993)).

5 With some drugs, it would be advantageous to effect administration *via* the oral route, which is accepted as one of the most convenient routes of administration, and for the drug to be released at a later, predetermined time following administration.

10 Examples of single unit (tablet or capsule) systems that can provide such a delayed release of drug are described in US 5,213,808 and US 5,342,624. Both of these delivery systems may provide pulsatile delivery of the drug into the gastrointestinal tract from where the drug can be absorbed into the systemic circulation. US 5,213,808 describes a multilayer composition providing constant release of drug followed by a pulse of release after a
15 pre-determined interval. The composition is not specifically designed to be retained in the stomach for prolonged periods. US 5,342,624 describes a device in which drug is retained within an insoluble shell by means of a swelling plug. The device, which is especially suitable for time-delayed oral release of drug in man, is not multiparticulate, nor is it designed for
20 prolonged retention in the stomach.

Such oral drug delivery systems may perform adequately, provided that the site of drug release in the intestines does not prejudice the subsequent absorption of the drug into the systemic circulation (in other words the
25 drug should be well absorbed from the different relevant regions of the gastrointestinal tract).

However, some drugs may be degraded if they are released into the acid contents of the stomach and some drugs are only absorbed from certain

preferred regions of the gastrointestinal tract. For example, drugs that exploit natural transport processes in the upper small intestine such as ACE inhibitors and levodopa, are known to be well absorbed at this site (the absorption window), but not in the distal small intestine or in the colon. Thus, once the drug delivery system has passed the absorption window, subsequent release of the agent may lead to poor systemic availability and, as a consequence, a poor therapeutic response.

An orally administered drug delivery system will be transported through the different regions of the gastrointestinal tract according to known physiological processes. The time that it will spend in the stomach, small intestines and colon depends upon factors such as whether the dosage form is taken with food (fasted versus fed) and the size of the dosage form (Davis *et al*, *Gut* 27, 886 (1986)). In normal human subjects, it is to be expected that a drug delivery system will pass into the colon after about 4 hours, following administration to the fasted stomach, and in about 8 hours following administration with a light meal.

Thus, the time delay/pulse release systems that are known from the prior art (and which are described above) may release drug at the correct time (as programmed into the delivery system), but at an inappropriate region of the gastrointestinal tract, depending on the feeding state of the patient. In other words, drug may be released into a region of the gastrointestinal tract where it is only poorly absorbed or not absorbed at all.

25

For drugs that are only absorbed from specific regions of the gastrointestinal tract, and for which time-delayed release is desirable, it would be advantageous, if it were possible, to "hold" a drug in the form of a drug delivery system above the preferred gastrointestinal tract region

of absorption, until such time as it is intended for the drug to be released. (For example, for drugs that are to be delivered to the small intestine, a system that was capable of retaining e.g. a pulsatile system above this region (e.g. in the stomach) for an extended period.)

5

Although gastroretentive systems are described in the art (for example swelling systems, bioadhesive systems, and floating systems that release the drug in a controlled manner (see for example, Moes, *Crit. Rev. Ther. Drug Carrier Syst.*, **10**, 143 (1993) and Deshpande *et al*, *Drug Dev. Ind. Pharm.*, **22**, 531 (1996))), such systems are not adapted to provide release of drug to a specific region of the gastrointestinal tract, since drug is released while the dosage form resides within the stomach.

Other published gastroretentive systems are also described in the art. Kawashima *et al* describe hollow microspheres for use as controlled release drug delivery systems that float in the stomach (*J. Pharm. Sci.*, **81**, 135 (1992)). Slow release granules, characterised by a low apparent density (0.65 g/mL) have been described by Lippold and Gunther (*Eur. J. Pharm. Biopharm.*, **37**, 254 (1991)). Chitosan-based granules for retention in the stomach have been described by Miyazaki *et al* (*Chem. Pharm. Bull.*, **36**, 4033 (1988)) and Inouye *et al* (*Drug Des. Deliv.*, **4**, 55, (1989)). Granules for retention in the stomach containing a gas generating layer comprising sodium bicarbonate and an organic acid are described in European patent application EP 235 718. Buoyant theophylline microspheres with controlled release properties have been described by Stithit *et al* (*J. Microencaps.*, **15**, 725 (1998)). In this document, microspheres are prepared by a modified emulsion-solvent evaporation method using a polymer mixture of cellulose acetate butyrate and Eudragit® RL 100 (1:1). Slow drug release is obtained over a period

of 12 hours in buffer systems with pH values of 1.2 and 7.5. None of these prior art documents describe enterically-coated floating and/or bioadhesive multiparticulate systems that specifically provide for e.g. pulsatile delivery of drug to a specific region of the gastrointestinal tract.

5

Floating coated ion-exchange resin beads loaded with bicarbonate have been shown to have gastroretentive properties by Atyabi *et al* (*J. Control. Rel.*, **42**, 105 (1996)). These authors suggested that anionic drugs could be accommodated by the system. Enteric coated beads for delivery to the small intestine are not described.

10

International patent application WO 98/52547 describes floating controlled release particulate systems that have gastroretentive properties. The system described comprises microspheres including an active ingredient in the inner core, a rate controlling layer of a water insoluble polymer and an outer layer of a bioadhesive agent in the form of a cationic polymer. The microspheres are designed to release drug while in the stomach. A multiparticulate system that floats in the stomach but which is enteric coated and releases the drug in the small intestine is not described.

15

US patent No. 5,972,389 describes a controlled release oral dosage form containing a plurality of particles of a solid-state drug dispersed in a swellable/erodible polymer such as polyethylene oxide. The swellable/erodible nature of this polymer apparently causes the particles to be gastroretentive. The gastric retained dosage form gradually erodes, releasing the drug in a controlled manner. Enteric coated drug particles are mentioned as a component of the controlled-release dosage form in order to provide site-specific delivery, but the particles themselves are not enterically-coated.

20

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There is a need for effective pharmaceutical formulations that release drug, for example in a pulsatile manner, to a region of the gastrointestinal tract (e.g. the small intestine and not the stomach) in a time-delayed
5 fashion.

We have found, surprisingly, that, in respect of delivery of drugs to the small intestine, the above-mentioned problems may be solved using a formulation comprising an enteric-coated floating multiparticulate system
10 that is retained in the stomach for a longer period of time than a conventional non-floating system. In such a system, no drug (i.e. less than 25% of the amount of drug in the formulation) is released in the acidic conditions of the stomach. Drug release begins when the formulation is exposed to conditions associated with the small intestine.
15 The system can additionally be coated with a bioadhesive material to further prolong retention within the stomach.

Thus, according to a first aspect of the invention there is provided a novel multiparticulate drug delivery composition (system) for the delivery of a
20 pharmacologically-active agent to the gastrointestinal tract of a mammal (e.g. the small intestine), wherein the particles of the composition are drug loaded, float on the stomach contents following oral administration and/or when suspended in less than or equal to 0.1 M hydrochloric acid, and are coated with an enteric polymer.

25

For the avoidance of doubt, the particles of the multiparticulate system (hereinafter referred to a "multiparticulates") are each individually drug loaded and are each provided with an enteric coating.

The medium upon which multiparticulates according to the invention float includes aqueous solvents, including pure water and aqueous hydrochloric acid of a strength of 0.1 M or less, and/or other aqueous liquids that approximate, in terms of their physical and chemical properties, to gastric
5 juices found in the human stomach.

By “pharmacologically-active agent”, we include substances that are capable of eliciting a therapeutic response in a mammal.

10 By “multiparticulate composition” or “multiparticulate system”, we include systems comprising a plurality of microspheres, microcapsules, microparticles, pellets, beads, small tablets or granules.

Suitable enteric polymers for use as the coating of the microparticulates
15 are well known to those skilled in the art and include those that prevent significant release of the incorporated drug into an acid environment (below pH 2), but that permit release of the drug in a more alkaline environment of pH 5.0 or greater. Such polymers thus include, but are not limited to, copolymers of methacrylic acid and methacrylic acid esters,
20 hydroxypropylmethylcellulose phthalate (HPMCP) and cellulose acetate phthalate. Copolymers of methacrylic acid and methacrylic acid esters are preferred. The Eudragits (registered trademark of Röhm Pharma, Darmstadt, Germany) are a family of polymers based on methacrylic acid. There are three types of Eudragit® which are copolymers of methacrylic
25 acid and methacrylic acid esters. These are Eudragit® S (S100/S12.5), Eudragit® L (L100/L12.5) and Eudragit® L100-55/L30D-55, which are all insoluble in acidic media, but which dissolve above pH 7.0, 6.0 and 5.5, respectively. The polymers are listed in the United States Pharmacopoeia/National Formulary as “Methacrylic Acid Copolymers

Type B, A and C", respectively. In accordance with the present invention, Eudragit® L and Eudragit® L100-55/L30D-55 are especially preferred, and Eudragit® L100-55/L30D-55 is most especially preferred.

- 5 The size of the multiparticulates may be from 0.1 to 5000 microns. A size from 0.5 to 2500 microns is preferred and a size from 1 to 1000 microns is most preferred.

The multiparticulates may also be provided with a layer of bioadhesive
10 material to provide adhesion to the stomach mucosa and further enhance retention in the stomach. Suitable bioadhesive materials include, but are not limited to, chitosan, chitosan derivatives, polygalactosamine, proteins (polyaminoacids) such as polylysine and polyornithine, polyquaternary compounds, prolamine, polyimine, dextran, diethylaminoethyl-dextran
15 (DEAE), DEAE-imine, polyvinylpyridine, polythiodiethylaminomethyl-ethylene (PTDAE), polyhistidine, DEAE-methacrylate, DEAE-acrylamide, poly-p-aminostyrene, polyoxethane, polymethacrylates (e.g. Eudragit® RL/RS), polyamidoamines, cationic starches or starch derivatives, cationic gelatin, gelatin, carbomer, carboxymethylcellulose,
20 pectin, carbopol, sodium carboxymethyl cellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, methylcellulose, sodium hyaluronate, guar gum, sodium alginate and polycarbophil.

Chitosan is the preferred choice for use as the bioadhesive material when
25 such a material is employed. Chitosan is a positively charged biopolymer at gastric pH. It is known that chitosan may interact with negatively charged sialic acid groups in mucin (Fiebrig *et al*, *Progress in Colloid and Polymers Sci.*, **94**, 66 (1994)). Chitosan is prepared by the deacetylation of chitin. In accordance with the present invention, the degree of

acetylation of chitosan should be greater than 40%, preferably greater than 60% and most preferably greater than 80%. The chitosan should preferably have a molecular weight in the range 10,000 to 1,000,000 Da, more preferably in the range 15,000 to 750,000 Da and most preferably in the range 20,000 to 500,000 Da. In the preparation of the compositions of the invention, chitosan is preferably applied as an aqueous solution. The aqueous solution may be prepared by dissolving chitosan base in an acid or by dissolving salt (e.g. glutamate, lactate, hydrochloride) in water. Alternatively, chitosan may be employed in the form of a derivative, such as N-trimethyl chitosan chloride.

The drug to be delivered with the drug delivery system of the invention may either comprise the cores of the multiparticulates, be contained within the body of those cores, or be coated onto the surface of those cores, once formed. The cores of the multiparticulate system, which may be prepared using a variety of pharmaceutically-acceptable materials known to those skilled in the art (some of which are commercially-available), such as lactose, starch and its derivatives (e.g. pre-gelatinised starch), mannitol, microcrystalline cellulose, stearic acid or pharmaceutically acceptable salts thereof, dicalcium phosphate, dextrose and/or sucrose (e.g. sugar spheres (non-pareils)), may be produced by various methods known to those skilled in the art such as granulation, freeze-drying, spray-drying, extrusion/spheronisation, tableting and/or spray-coating.

Depending upon the method that is used to form the cores of the multiparticulates, they may comprise other excipients, such as binding agents. Such binding agents, which may be used to cohere the cores (with or without drug) include, but are not limited to, polyethylene glycols (PEGs), polyvinylpyrrolidone, acacia, carboxymethylcellulose,

hydroxyethylcellulose, gelatin, starch, dextrose, glucose and hydroxypropylmethylcellulose. When drug is coated onto the cores once the latter are formed, it may be applied in the presence of a suitable binding agent, including those mentioned above.

5

The amount of drug in the microparticulate system can be from 0.1% to 90% by weight, more preferably from 0.5% to 80% by weight and most preferably from 1% to 70% by weight of the total weight of the system (including the enteric coating and any other excipients (e.g. bioadhesive) that are employed).

10

The enteric coating is applied to the multiparticulates in order to prevent the drug contained within from being released in the acidic environment of the stomach. The enteric polymer may be formulated as a solution with appropriate additional excipients, such as plasticisers (e.g. dibutyl sebacate, triethyl citrate) and anti-tack agents (e.g. talc, kaolin, glycerol monostearate). Methods of formulating enteric polymers, including the selection of solvents and the type and quantity of additional excipients are well known to those skilled in the art.

15

Enteric polymers may typically be applied onto the multiparticulates using a spray-coating process and using standard pharmaceutical coating equipment. Manufacturers of such equipment include Aeromatic-Fielder (Eastleigh, UK), Glatt, (Binzen/Lörrach, Germany), BWI Manesty (Speke, UK) and Freund (Tokyo, Japan).

20

The quantity of enteric coating that is applied needs to be sufficient to prevent significant drug release while the multiparticulate dosage form resides within the stomach. By "preventing significant drug release"

under these conditions, we include that less than 25%, more preferably less than 20%, and most preferably less than 15%, of the drug contained within the multiparticulates is released after 2 hours exposure to 0.1 M hydrochloric acid using the standard pharmacopoeial dissolution test procedure (United States Pharmacopoeia method II; European Pharmacopoeia Paddle Method). The quantity of coating needed to achieve this level of protection will vary and will depend on the size, shape and surface characteristics of the multiparticulates. For example, large, spherical, smooth multiparticulates will require a lower level of coating than small, irregular particles with a rough surface. As a guideline, for Eudragit® enteric polymers, the manufacturer indicates that a polymer coat thickness of 30 to 50 microns (equivalent to 4 to 6 mg polymer/cm²) is sufficient to provide resistance to drug release in the stomach (technical literature produced by Röhm Pharma, Darmstadt, Germany). In any event, the amount of enteric coating may be determined routinely by the skilled person.

If included, bioadhesive material may be applied as a layer onto the enteric coating. Such a bioadhesive layer may be applied to the multiparticulates from solution by using a spray-coating technique or, alternatively, mixed in a finely-divided powder form with the particles.

An essential feature of this invention is that the enteric coated multiparticulates float. By the term "float", we include that when 0.5 g to 1.0 g of multiparticulates are added to a beaker containing 500 mL of, for example, 0.1 M hydrochloric acid (to simulate the pH conditions in the stomach), at least 25%, preferably at least 33% and most preferably at least 50% remain floating on the liquid surface after a period of 30 minutes.

Floating properties may be conferred on the mutiparticulates by three means: (a) using particles of a low density, (b) employing a hydrophobic outer coating, or (c) employing an outer coating comprising chitosan
5 applied as a powder.

By "particles of low density" we include particles which, when coated with an enteric polymer alone (in the absence of any additional coating) float according to the definition described hereinbefore. The skilled
10 person will appreciate that systems that provide these properties may be devised in multiparticulates without recourse to inventive input. For example, such particles may be prepared by utilising materials that have an inherently low density or by producing porous particles in which a gas, for example air, is trapped by means of an impermeable membrane.

15 Flotation may also be conferred upon an otherwise non-floating multiparticulate system by means of employing a hydrophobic outer coating, which coating provides the resultant multiparticulates with a contact angle, as between the particle surface and the relevant liquid
20 surface, of greater than 0 degrees. The concept of contact angles is well known in the art. Further details may be found in for example *Physicochemical Principles of Pharmacy*, Florence and Attwood (eds.), 3rd edition, Macmillan, London (1998). A preferred such hydrophobic outer coating is stearic acid or a salt thereof.

25 Surprisingly, we have also found that floating properties may also be conferred upon an otherwise non-floating multiparticulate system by means of blending enteric-coated multiparticulates with chitosan in the form of a finely divided (mean particle size below 100 μm) powder such

that the powder adheres to the surface of the multiparticulates by means of electrostatic interaction. Chitosan may be used in the form of the free base, a salt thereof or a derivative thereof.

- 5 In one preferred embodiment of the invention, the multiparticulates comprise enteric coated cores that are non-floating as such. Suitable cores include enteric coated granules, enteric coated pellets made by the process of extrusion/spheronisation and enteric coated particles of pure drug. Preferred non-floating cores are enteric coated non-pareils. Non-pareils
10 are spheres primarily comprising sucrose that are available commercially in diameters ranging from 425 to 1400 microns. Suppliers include NP Pharm (Bazainville, France) and Crompton & Knowles (Pennsauken, USA). Layers of drug and enteric polymer are applied in sequence by spray-coating processes. Optionally, a bioadhesive coating may be
15 applied onto the enteric polymer layer. The preferred bioadhesive is chitosan. At least 1%, more preferably at least 2% and most preferably at least 3% by weight of the multiparticulate composition is comprised of the outer bioadhesive layer. Floating properties may be achieved by one of three means; by applying an additional layer of stearic acid onto the outer
20 enteric or bioadhesive layer; by blending the enteric coated cores with chitosan; or by incorporating stearic acid into the outer bioadhesive coat.

By the term "stearic acid", we include the free acid or a pharmaceutically acceptable salt thereof. Preferred forms of stearic acid include
25 magnesium stearate.

Where floating properties are achieved by applying an additional layer of stearic acid onto the enteric or bioadhesive layer, this layer may be applied by methods including, but not restricted to, blending and spray

coating. For example, stearic acid and the particles may be combined in a pharmaceutical mixer, such as a V-blender or Turbula® mixer, such that the stearic acid adheres to the surface of the multiparticulates as a thin layer. Alternatively, stearic acid may be suspended or dissolved in a suitable solvent and sprayed on to the multiparticulates, whereby evaporation of the solvent deposits the stearic acid on to the surface of the particles.

Where floating properties are achieved by blending with chitosan, the chitosan and enteric coated multiparticulate are combined by means of a pharmaceutical mixer, as described hereinbefore. The chitosan content of the final composition is in the range 0.1% to 80% by weight, more preferably 0.5% to 65% by weight and most preferably 1 to 50% by weight.

Achieving floating properties by use of a coating comprising a mixture of chitosan and stearic acid may be advantageous. Such a combination produces both a prolongation of floating and a prolongation of bioadhesion, the latter a result of the increased hydrophobicity of the coat leading to a slowing in chitosan dissolution rate. Such a mixture may be applied to the multiparticulate by spray coating in which the stearic acid and chitosan are dissolved or suspended in an appropriate solvent or by blending a mixture of the two powders with the multiparticulate. The mixture of chitosan and stearic acid preferably constitutes 0.1% to 80% by weight of the final composition, more preferably 0.5% to 65% by weight and most preferably 1 to 50% by weight. The chitosan content of the chitosan/stearic acid mixture is in the range 1 to 99% by weight, more preferably 5 to 95% and most preferably 10 to 90%.

In a second preferred embodiment of the invention, the multiparticulates are based upon low density pellet cores comprising stearic acid, to which drug and enteric polymer are applied by a spray coating process. Optionally, a layer of bioadhesive material, preferably chitosan, may be applied onto the enteric coating. Optionally, a further increase in the duration of floating may be achieved by the means described hereinbefore, namely by applying an additional layer of stearic acid onto the enteric or bioadhesive layer; by blending the enteric coated cores with chitosan; by incorporating stearic acid into the outer bioadhesive coat. The core of the pellet may be prepared by binding stearic acid using an appropriate binding agent or, in the case of the free acid form of stearic acid, by the use of heat. Suitable binding agents include, but are not limited to, polyethylene glycols (PEGs), polyvinylpyrrolidone, acacia, carboxymethylcellulose, hydroxyethylcellulose, gelatin and starch. The preferred binding agent is polyethylene glycol. The preferred molecular weight of polyethylene glycol is in the range 1,000 Da to 20,000 Da. Optionally, additional excipient may be incorporated with the binder and stearic acid or salt thereof for the purpose of increasing pellet hardness and reducing pellet friability. Suitable excipients include lactose, starch and its derivatives (e.g. pre-gelatinised starch), mannitol, microcrystalline cellulose, stearic acid or pharmaceutically acceptable salts thereof, dicalcium phosphate, dextrose and/or sucrose. A variety of known methods may be used to prepare the pellets, including hot-melt granulation, spray granulation and extrusion/spheronisation. The uncoated pellet core should preferably comprise at least 30%. More preferably at least 40% and most preferably at least 50% by weight of stearic acid. In the final composition containing drug, enteric coating and, optionally, bioadhesive or hydrophobic layer, the core may preferably comprise at least 40%, more preferably at least 50% and most

preferably at least 60% by weight of the multiparticulate system; the enteric coating may preferably comprise between 1 and 35%, more preferably between 2 and 30% and most preferably between 5 and 25% by weight of the multiparticulate system; the bioadhesive coating (if employed) may preferably comprise between 0.1 and 50%, more preferably between 0.2 and 40% and most preferably between 0.5 and 30% by weight of the multiparticulate system; the hydrophobic coating (if employed) may comprise between 0.1 and 25%, more preferably between 0.2 and 20% and most preferably between 0.5 and 15% by weight of the multiparticulate system; and the drug may comprise between 1 and 60%, more preferably between 1 and 50% and most preferably between 1 and 40% by weight of the multiparticulate system.

In a third preferred embodiment of the invention, the multiparticulates are based upon low density granules prepared by freeze drying. The drug may be incorporated into the granule or applied as a layer onto the completed granule by spray coating. The granules with incorporated or coated drug are further coated with enteric polymer and, optionally, bioadhesive material. Optionally, a further increase in the duration of floating may be achieved by the means described hereinbefore. Methods for preparing granules are well known to those skilled in the art (see e.g. *Pharmaceutical Dosage Forms: Tablets*, Volumes 1 and 2 (Leiberman, Lachman and Schwartz (eds.), Marcel Dekker, New York (1990))). The materials suitable for making the freeze-dried granules include, but are not limited to lactose, starch and derivatives (e.g. pre-gelatinised starch), mannitol, dextrose, sucrose and microcrystalline cellulose. Binding agents for incorporation into the granules include, but are not limited to, polyethylene glycols (PEGs), polyvinylpyrrolidone, acacia, carboxymethylcellulose, hydroxyethylcellulose, gelatin and starch. If

drug is to be incorporated into the granule, it may be added as a solution or in powder form. In the final composition containing drug, enteric coating and, optionally, bioadhesive layer, the freeze-dried granule core (containing drug at between 0.1 and 99.9%, preferably between 1 and 5 95% and more preferably 2 to 90% by weight of the core) should preferably comprise at least 40%, more preferably at least 50%, and most preferably at least 60% by weight of the multiparticulate system; the enteric coating may preferably comprise between 1 and 35%, more preferably between 2 and 30% and most preferably between 5 and 25% by 10 weight of the multiparticulate system; the bioadhesive coating (if employed) may preferably comprise between 0.1 and 50%, more preferably between 0.2 and 40% and most preferably between 0.5 and 30% by weight of the multiparticulate system; the hydrophobic coating (if employed) may comprise between 0.1 and 25%, more preferably between 15 0.2 and 20% and most preferably between 0.5 and 15% by weight of the multiparticulate system.

The compositions of the invention may be administered to a mammal in suitable dosage forms, in accordance with techniques, and *via* delivery 20 devices, all of which are known to those skilled in the art, for example by way of a capsule, a powder or as a compressed tablet, administered by mouth, that dissolves in the stomach to release the multiparticulates. The compositions may be administered with a suitable dosing liquid (e.g. water).

25

Drugs that display preferential absorption from the upper small intestine are suitable for use in the compositions of the invention and such drugs can be found in all therapeutic categories. A non-exclusive list is as follows: levodopa, methyldopa, frusemide; ACE inhibitors, such as

carvedilol, captopril, cilazipril, enalapril, fosinopril, lisinopril, moexipril, perindopril (preferably), ramipril and trandolapril; atenolol, topiramate, hydrochlorothiazide, orlistat, alendronic acid, disodium etidronate, disodium pamidronate, sodium clodronate, tiludronic acid.

5

Combinations of the above-mentioned drugs may also be employed.

Although the compositions of the invention find particular utility in the pulsatile delivery of drugs to the small intestine (by which we include that
10 all of the drug is released within 30 minutes once exposed to the more alkaline environment associated with the small intestine), they are also suitable for providing delayed local delivery of drugs that are useful for the treatment of diseases affecting the small intestine. Examples include the delivery of anti-inflammatory agents into the small intestine for the
15 topical treatment of Crohn's disease. Suitable anti-inflammatory agents may thus include corticosteroids, such as hydrocortisone, prednisolone and mesalamine (5-aminosalicylic acid). In such instances, it may further be advantageous for the multiparticulates to provide for slow release of drug within the small intestine, in order to make the drug available for topical
20 application throughout the whole of the small intestine. Slow release from each multiparticulate may be achieved by application of a rate-controlling polymer membrane, such as ethylcellulose, beneath the enteric coating, or by preparing the core of the multiparticulate from materials that provide controlled release, such as hydroxypropylmethylcellulose. Suitable
25 techniques for preparing slow release formulations are well known in the art (see, for example Lachman *et al* (eds.) *infra*, Cole *et al* (eds.), *Pharmaceutical Coating Technology*, Taylor & Francis, Chichester (1995)).

Thus, the compositions of the invention may be used to treat/prevent diseases/conditions in mammalian patients depending upon the drug(s) which is/are employed. For the above, non-exhaustive, lists of drugs, diseases/conditions which may be mentioned include those against which the therapeutic agent(s) in question are known to be effective, and include those specifically listed for the drugs in question in Martindale, "*The Extra Pharmacopoeia*", 31st Edition, Royal Pharmaceutical Society (1996).

The amount of drug which may be employed in the compositions according to the invention will depend upon the agent which is used, and the disease to be treated, but may be in the range 0.01 mg to 1 g. However, it will be clear to the skilled person that suitable doses of drugs can be readily determined non-inventively. For example, estimates of dosage can be made from known injectable products assuming that from 0.1 to 100% of the dose is absorbed. Suitable daily doses are in the range 0.01 mg to 5 g/day depending upon the drug(s) which is/are employed.

The compositions according to the invention may be dosed once, or more (eg three) times, daily depending on the condition to be treated.

Compositions according to the invention have the advantage that they may provide a delayed (and in certain cases pulsatile) release of drug to specific regions of the gastrointestinal tract in mammalian (e.g. human) subjects.

Moreover, compositions of the invention also have the advantage that they may be prepared using established pharmaceutical processing methods and

employ materials that are approved for use in foods or pharmaceuticals or of like regulatory status.

According to a further aspect of the invention there is provided a method
5 of treatment or prophylaxis of a disease which comprises administration of a composition according to the present invention including a drug which is effective against said disease to a patient in need of such treatment or prophylaxis.

10 The invention is illustrated, but in no way limited, by the following examples, in which:

Figure 1 shows the release of perindopril from floating enteric-coated magnesium stearate-based pellets during a dissolution test carried out in
15 respect of a formulation prepared in accordance with Example 1.

Figure 2 shows the release of perindopril from floating freeze-dried granules coated with enteric polymer and chitosan during a dissolution test carried out in respect of a formulation prepared in accordance with
20 Example 2.

Figure 3 shows the release of perindopril from floating non-pariel seeds coated with enteric polymer and chitosan/magnesium stearate during a dissolution test carried out in respect of a formulation prepared in
25 accordance with Example 3.

Example 1Floating Microparticles Based on Magnesium Stearate/Starch/Polyethylene Glycol Pellets

300 g of magnesium stearate (Fisher Scientific) and 50 g of Starch 1500®
5 (Colorcon, Orpington, UK) were placed into the bowl of a domestic food
processor (Braun Model Combimax 600, Frankfurt, Germany). 200 g of
polyethylene glycol (PEG) 8000 (Sigma) was melted by heating to
approximately 70°C. The powders were mixed at speed setting "8" and
the melted PEG added. The resulting pelletised mass of material was
10 passed through sieves and a size fraction between 0.25 and 0.65 mm
collected.

20 g of hydroxypropylmethylcellulose (Methocel® grade E5, Colorcon)
was dissolved in 150 mL of isopropanol and then 50 mL of water added.
15 6 g of perindopril (Technologie Servier, Orleans, France) was then
dissolved in this solution. This solution was spray coated onto the
magnesium stearate/starch/PEG pellets using an Aeromatic STREA-1
fluidised bed coater (Niro-Aeromatic, Budendorf, Switzerland) (coating
conditions: inlet temperature, 40°C; atomisation pressure 1 bar; spray rate
20 15 mL/min).

312 mL of isopropanol (Fisher, Loughborough, UK) was mixed with 8
mL of water. Into 212 mL of this solvent mixture was stirred 22 g of
Eudragit® L100-55 (Rohm Pharma, Darmstadt, Germany). Into the
25 remaining solvent mixture was dispersed 2.7 g of magnesium stearate
(Fisher) and 2.7 g of talc (Janssen Chimica, Belgium). When the
Eudragit® L100-55 had dissolved, 4.2 g of dibutyl sebacate (Morflex,
Greensboro, USA) and the magnesium stearate/talc suspension were

added. This enteric coating mixture was applied to the magnesium stearate/starch/PEG pellets using the Aeromatic STREA-1 coater (coating conditions: inlet temperature 35°C; atomisation pressure 1 bar; spray rate approx. 15 mL/min).

5

Floating test: 1 gram of coated particles were sprinkled onto 500 mL of 0.1 M hydrochloric acid in a 150 mL beaker. After 30 minutes, in excess of 75% of the granules were still floating on the liquid surface.

- 10 Dissolution test: The dissolution performance of the coated pellets was assessed using a modification of USP Test Method II. 500 g of coated particles (equivalent to 4 mg of perindopril) were added to 375 mL of 0.1 M hydrochloric acid (37°C) which was agitated by paddle at 50 rpm. At 0, 15, 30 and 60 minutes, a 4 mL of dissolution medium was removed
- 15 from the vessel and assayed for perindopril content using a reverse-phase HPLC method. After 1 hour, 125 mL of 0.2M tribasic sodium phosphate buffer was added to each dissolution vessel and the pH adjusted to 6 with a few drops of 0.1M hydrochloric acid. Samples of dissolution medium were then withdrawn at 65, 75 and 105 minutes. The result of this
- 20 dissolution test is shown in Figure 1 (percentage of released perindopril vs. time).

Example 2

Floating Microparticles Based on Freeze-Dried Lactose Granules

- 25 10 g of polyvinylpyrrolidone (Sigma, Poole, UK) was dissolved in 100 mL of water. 1.6 g of perindopril was dissolved in the povidone solution. 200 g of spray-dried lactose ("Zeparox", Borculo, Chester, UK) was placed into the bowl of a domestic food mixer (Kenwood, Havant, UK). A K-shaped mixing arm was attached to the mixer and the mixer started

on minimum speed. All of the solution containing perindopril was slowly mixed into the lactose. The resulting lactose granules were passed through a 0.5 mm sieve and transferred into a plastic tray. The granules were frozen by placing the tray into liquid nitrogen. The frozen granules
5 were then transferred into a laboratory-scale freeze drier (Edwards Modulyo, Crawley, UK) and freeze-dried for a period of 36 hours.

577 mL of isopropanol (Fisher) was mixed with 13 mL of water. Into 490 mL of this solvent mixture was stirred 40 g of Eudragit® L100-55. Into
10 the remaining solvent mixture was dispersed 10 g of magnesium stearate (Fisher Scientific). When the Eudragit® L100-55 had dissolved, 7.7 g of dibutyl sebacate (Morflex Inc, Greensboro, USA) and the magnesium stearate suspension were added to form the enteric coating solution. The coating mixture was applied to the granules using the Aeromatic STREA-
15 1, as described in Example 1.

5 g of chitosan glutamate (Protasan® UPG213, Pronova, Drammen, Norway) was dissolved in 400 mL of water. 3 g of magnesium stearate was dispersed in 5 mL of ethanol and mixed into the chitosan solution.
20 This mixture was spray coated onto the above enteric-coated perindopril granules using the Aeromatic STREA-1 (coating conditions: inlet temperature 60°C; atomisation pressure 1 bar; spray rate 15 mL/min).

The particles floated when added to a beaker containing 0.1M
25 hydrochloric acid. The dissolution performance of the coated particles was assessed using the method described in Example 1. Dissolution test results are presented in Figure 2.

Example 3Floating Microparticles Based on Coated Sugar Spheres

Perindopril/HPMC solution was prepared as described in Example 1 and spray-coated onto 200 g of 500-600 μm diameter sugar pellets (“non-
5 pareils”) (NP Pharm, Bazainville, France) using the process conditions described in Example 1.

577 mL of isopropanol was mixed with 13 mL of water. Into 490 mL of this solvent mixture was stirred 60 g of Eudragit® L100-55. Into the
10 remaining solvent mixture was dispersed 15 g of magnesium stearate. When the Eudragit® L100-55 had dissolved, 11.6 g of dibutyl sebacate and the magnesium stearate suspension were added. This mixture was applied to the coated sugar pellets, as described in Example 1.

15 5 g of chitosan glutamate was dissolved in 400 mL of water. 5 g of magnesium stearate were dispersed in 5 mL of ethanol and mixed into the chitosan solution. This mixture was spray coated onto the above enteric-coated sugar pellets as described in Example 2.

20 The particles floated when added to a beaker containing 0.1M hydrochloric acid. The dissolution performance of the coated particles was assessed using the method described in Example 1. Dissolution test results are presented in Figure 3.

Example 4Floating Pellets Based on Magnesium Stearate/Starch/Polyethylene Glycol Pellets Dry Blended with Magnesium Stearate

Floating pellets were prepared as described in Example 1. 5 g of the
5 finished pellets were added to 50 mg of magnesium stearate in a 200 mL
bottle and mixed together for 5 minutes using a Turbula mixer TC2 (Glen
Creston, Stanmore, UK) at medium speed setting.

The floating ability of the resulting magnesium stearate-coated pellets was
10 assessed by placing 1 g of the microparticles into a beaker containing 500
mL of 0.1 M HCl which was stirred gently with a magnetic stirrer bar.
All of the particles remained floating on the liquid surface after 5 hours.

Example 5

15 Floating Microparticles Based on Coated Sugar Spheres Blended with
Chitosan

Enteric coated sugar spheres, with an outer coating comprising Eudragit
L100-55, dibutyl sebacate and magnesium stearate, are prepared as
described in Example 3. 10 g of the coated spheres and 1 g of chitosan
20 glutamate are weighed into a glass bottle. The bottle contents are mixed
together for 10 minutes using a Turbula TC2 mixer. When a sample of
the sugar spheres/chitosan mixture is applied to the surface of a beaker
containing 500 mL of 0.1 M HCl, the sugar spheres float.

25 Example 6

Floating Pellets to Provide Controlled Delivery of 5-Aminosalicylic acid
into the Small Intestine

500 g of 5-aminosalicylic acid (Sigma), 200 g of microcrystalline cellulose
(Avicel PH102) and 300 g of hydroxypropylmethylcellulose (Methocel®

- K100M) are mixed together using a domestic food mixer. A solution of 10 g polyvinylpyrrolidone dissolved in 100 mL of ethanol is slowly added to the powders while mixing continues. The powder mass is passed through a 1 mm sieve. The resulting coarse granules are spread onto a tray and dried in an oven at 40°C for 2 hours. The dried granules are lightly milled using a pestle and mortar and screened through a 0.5 mm sieve. An enteric coating solution is prepared as described in Example 2 comprising isopropanol, water, Eudragit® L100-55, dibutyl sebacate and magnesium stearate. This solution is applied to the screened granules using the Aeromatic STREA-1 coater. 10 g of coated granules are mixed with 1 g of chitosan glutamate, as described in Example 5. When a sample of the granule/chitosan mixture is applied to the surface of a beaker containing 500 mL of 0.1 M HCl, the granules float.
- The dissolution performance of a 1 g sample of the granule/chitosan mixture is measured using the USP II method, as described in Example 1. The release of 5-aminosalicylic acid from the pellets is monitored by UV analysis at 330 nm. During 1 hour of exposure to acid conditions, no 5-aminosalicylic acid is released. When the pH of the dissolution medium is changed to pH 6, as described in Example 1, release of 5-aminosalicylic acid from the granules begins within 15 minutes. Around 50% of the 5-aminosalicylic acid is released after 1 hour. All 5-aminosalicylic acid is released within 2 hours.

Claims

1. A multiparticulate drug delivery composition adapted for the
5 delivery of a pharmacologically-active agent to the small intestine
of a mammal, including drug-containing coated particles, which
particles comprise a drug-containing core coated with an enteric
polymer, wherein the particles float on the stomach contents
following oral administration.
- 10 2. A multiparticulate drug delivery composition adapted for the
delivery of a pharmacologically-active agent to the small intestine
of a mammal, including drug-containing coated particles, which
particles comprise a drug-containing core coated with an enteric
15 polymer, wherein the particles float when suspended in an aqueous
solution comprising less than or equal to 0.1 M hydrochloric acid.
3. A composition as claimed in Claim 1 or Claim 2 wherein the drug-
containing particles of the multiparticulate system are coated with
20 an outer layer of bioadhesive material.
4. A composition as claimed in any one of the preceding claims
wherein the cores of the multiparticulate system are freeze-dried
granules.

5. A composition as claimed in any one of Claims 1 to 3 wherein the cores of the multiparticulate system comprise pellets containing stearic acid or a pharmaceutically acceptable salt thereof.
- 5 6. A composition as claimed in Claim 5 wherein the pharmaceutically acceptable salt of stearic acid is magnesium stearate.
7. A composition as claimed in Claim 5 or Claim 6 wherein the stearic acid or pharmaceutically acceptable salt thereof comprises at least
10 30% by weight of the uncoated pellet core.
8. A composition as claimed in any one of Claims 1 to 3 wherein the multiparticulate system is based on coated non-pareil spheres.
- 15 9. A composition as claimed in any one of Claims 1 to 8 wherein the enteric polymer coating is selected from a copolymer of methacrylic acid and methacrylic acid esters, hydroxypropylmethylcellulose phthalate or cellulose acetate phthalate.
- 20 10. A composition as claimed in Claim 9 wherein the enteric coating is a copolymer of methacrylic acid and methacrylic acid esters.
11. A composition as claimed in any one of Claims 3 to 10 wherein the
25 outer bioadhesive material is chitosan, a salt thereof or a derivative thereof.

12. A composition as claimed in Claim 11 wherein the chitosan, salt or derivative is applied as a finely divided powder by blending.
- 5 13. A drug delivery composition as claimed in Claim 11 or Claim 12 wherein the outer bioadhesive coating layer also comprises stearic acid or a pharmaceutically acceptable salt thereof.
- 10 14. A composition as claimed in any one of Claims 1 to 13 wherein a hydrophobic coating is applied onto the enteric coating or the bioadhesive coating.
- 15 15. A composition as claimed in Claim 14 wherein the coating is stearic acid or a pharmaceutically acceptable salt thereof.
16. A composition as claimed in Claim 15 wherein the pharmaceutically acceptable salt of stearic acid is magnesium stearate.
- 20 17. A composition as claimed in Claim 15 or Claim 16 wherein the acid or salt is applied as a powder by blending.
- 25 18. A composition as claimed in any one of the preceding claims which further comprises a means of providing slow release of drug in the small intestine.

19. A method of administering a drug to the gastrointestinal tract, which comprises administering a composition according to any one of the previous claims to a patient.
- 5 20. A method of treatment or prophylaxis of a disease which comprises administration of a composition as claimed in any one of Claims 1 to 18 including a pharmacologically-active agent which is effective against said disease to a patient in need of such treatment or prophylaxis.
- 10 21. The use of a composition according to any one of Claims 1 to 18 for the manufacture of a medicament for the delivery of a pharmacologically-active agent to the gastrointestinal tract of a patient.
- 15 22. A composition according to any one of Claims 1 to 18 for use in the delivery of a pharmacologically-active agent to the gastrointestinal tract of a patient.

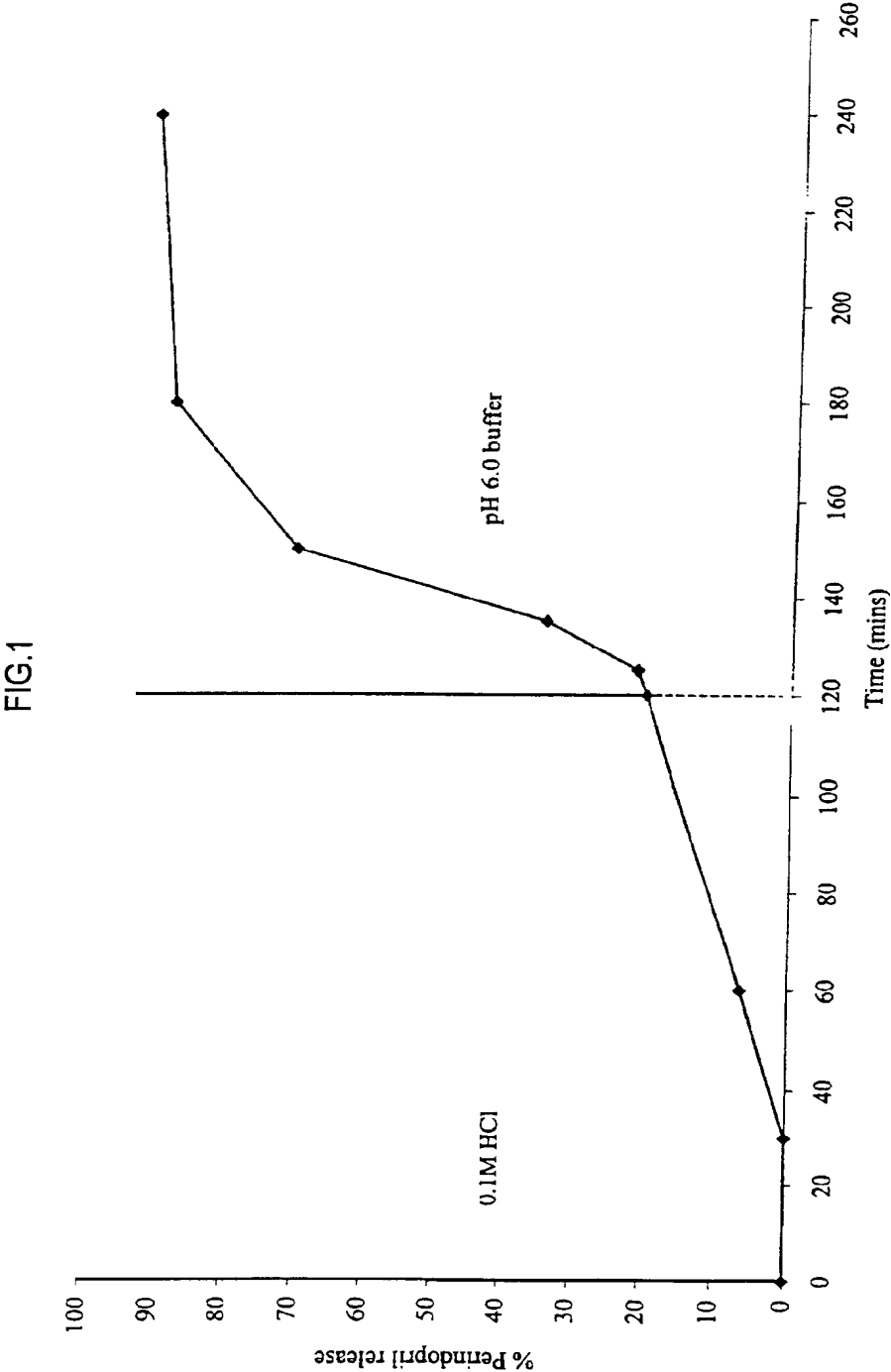
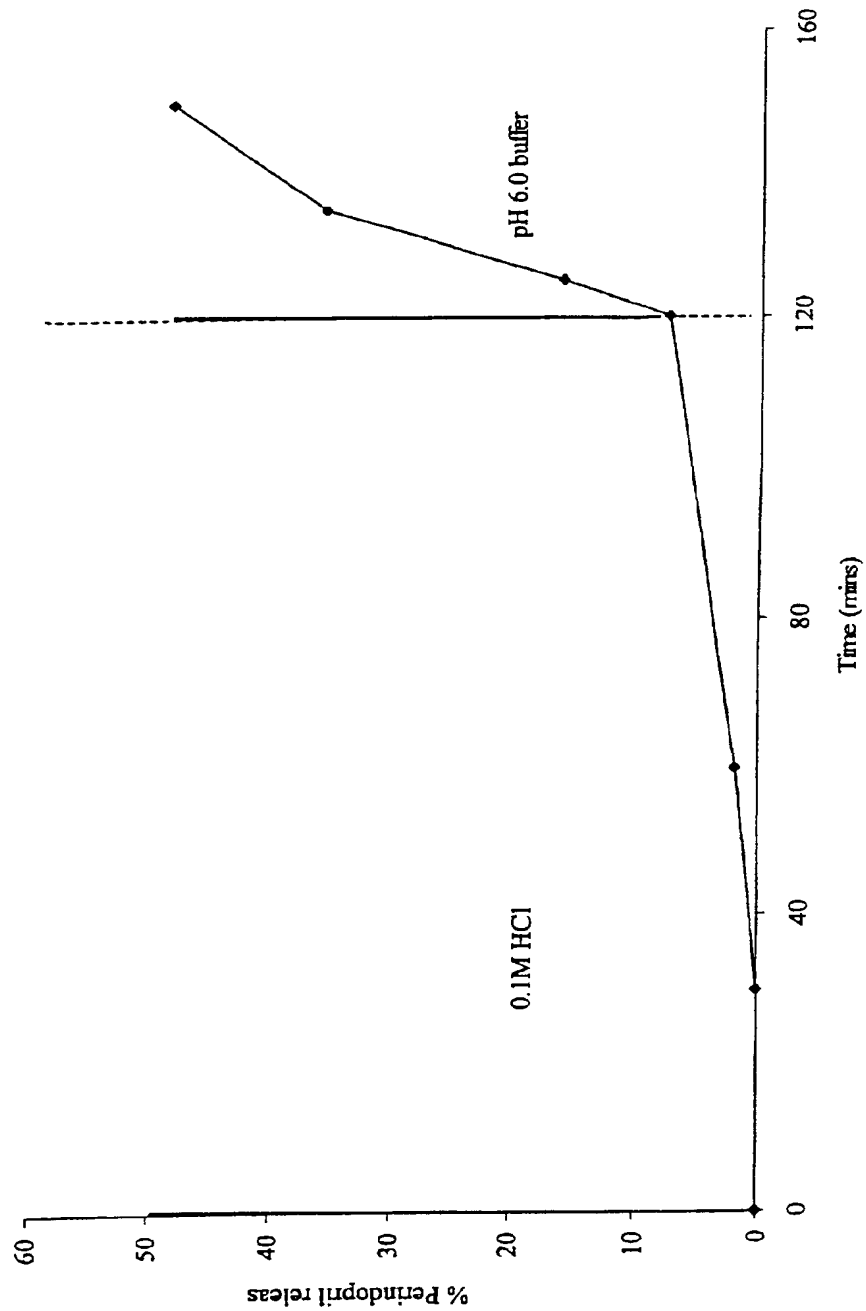
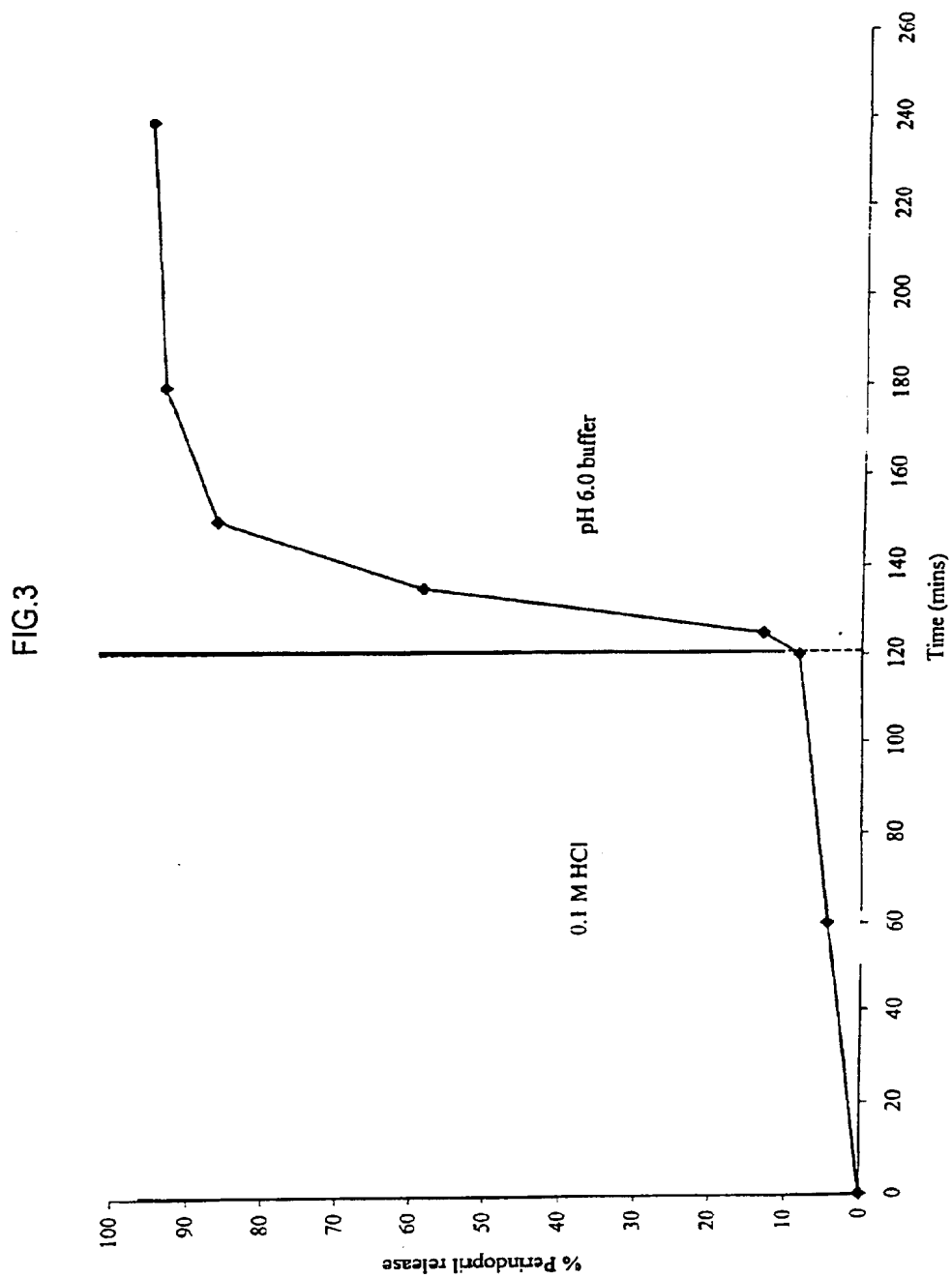


FIG.2



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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 01/00493

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00 A61K9/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BE 685 228 A (HOFFMANN-LA ROCHE) 8 February 1967 (1967-02-08) claims 1,2 page 17; examples A-D ---	1-3,8, 18-22
X	EP 0 200 902 A (FUJISAWA) 12 November 1986 (1986-11-12) examples 1,3 page 4, line 1 - line 10 ---	1-3, 8-10, 18-22
X	US 4 764 380 A (ALZA) 16 August 1988 (1988-08-16) column 3, line 37 - line 50 figures 1-4 column 3, line 66 - column 4, line 6 column 4, line 25 - line 50 column 8, line 5 - line 24 --- -/--	1,2,8, 18-22

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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PCT/GB 01/00493

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 235 718 A (EISAI) 9 September 1987 (1987-09-09) examples 1-3 ----	1,2,5-9, 18-22
X	WO 99 01112 A (ASTRA AKTIEBOLAGET) 14 January 1999 (1999-01-14) claims 1,6 page 9, line 16 - line 24 page 15; example 2 -----	1,2, 8-10, 18-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat. Application No

PCT/GB 01/00493

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
BE 685228 A	08-02-1967	NL 6611190 A US 3365365 A	10-02-1967 23-01-1968
EP 200902 A	12-11-1986	AT 67930 T AU 590255 B AU 5478386 A CA 1275045 A DE 3681755 A DK 136186 A JP 2035010 C JP 7072143 B JP 61233632 A US 4690822 A	15-10-1991 02-11-1989 02-10-1986 09-10-1990 07-11-1991 27-09-1986 28-03-1996 02-08-1995 17-10-1986 01-09-1987
US 4764380 A	16-08-1988	US 4434153 A US 4649043 A US 4851232 A US 4642233 A US 4659558 A	28-02-1984 10-03-1987 25-07-1989 10-02-1987 21-04-1987
EP 235718 A	09-09-1987	JP 62195323 A AT 68969 T CA 1288697 A DE 3774148 A ES 2051698 T GR 3003031 T KR 9104578 B US 4844905 A	28-08-1987 15-11-1991 10-09-1991 05-12-1991 01-07-1994 17-02-1993 06-07-1991 04-07-1989
WO 9901112 A	14-01-1999	AU 8135798 A EP 1014952 A	25-01-1999 05-07-2000